

<110> Korea Research Institute of Bioscience and Biotechnology

<120> A novel Hansenula polymorpha gene coding for alpha 1,6 mannosyltransferase and process for the production of recombinant glycoproteins with Hansenula polymorpha mutant strain deficient in the same gene

<160> 14

<170> KopatentIn 1.71

<210> 1

<211> 1351

<212> DNA

<213> Hansenula polymorpha

<220>

<221> CDS

<222> (10)..(1293)

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Met Val Tyr Phe Leu Asn Phe Met Ser Ile Thr Asn Val Pro
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gtg ctg aag cgc gcg cga ctc tac atg gcg acg aat cgc cgg ctg gtg 99
Val Leu Lys Arg Ala Arg Leu Tyr Met Ala Thr Asn Arg Arg Leu Val
15 20 25 30

gtt gtt ctt gtg gtg ctg ctg tac tgg gtg gtc cag aac gtt tgg acg 147
Val Val Leu Val Val Leu Leu Tyr Trp Val Val Gln Asn Val Trp Thr
35 40 45

tgg agc cct ggg acg cgc gat ttg gcc caa gtg gac gcg aag atc gag 195
Trp Ser Pro Gly Thr Arg Asp Leu Ala Gln Val Asp Ala Lys Ile Glu
50 55 60

gcc gag cta aac tcg aat cta cat act ttt gga gcg cat ttg cgc cac 243
Ala Glu Leu Asn Ser Asn Leu His Thr Phe Gly Ala His Leu Arg His
65 70 75

tta aac cgg ctt cgg gca gag tcg gcc acc ctg cgt gaa aaa ctc acc 291
Leu Asn Arg Leu Pro Ala Glu Ser Ala Thr Leu Arg Glu Lys Leu Thr
80 85 90

ttc tat ttc cca tat tat cct gaa aag ccc gtg ccg aac cag atc tgg 339
Phe Tyr Phe Pro Tyr Tyr Pro Glu Lys Pro Val Pro Asn Gln Ile Trp
95 100 105 110

cag aca tgg aag gtc gat ctc gaa gac gac aac ttc ccc aag cag tac 387
Gln Thr Trp Lys Val Asp Leu Glu Asp Asp Asn Phe Pro Lys Gln Tyr
115 120 125

aga cgg ttt cag aag acg tgg gtc gag aaa aat cca gac tac gtg tac 435
Arg Arg Phe Gln Lys Thr Trp Val Glu Lys Asn Pro Asp Tyr Val Tyr
130 135 140

cac ctg att ccg gac tct gtg att gag gac ttt gtg gcg agt ttg tac 483
His Leu Ile Pro Asp Ser Val Ile Glu Asp Phe Val Ala Ser Leu Tyr
145 150 155

gcg aac gtg ccg gag gtg gtc aga gcg tac cag ctg ctt ccg aaa aat 531
Ala Asn Val Pro Glu Val Val Arg Ala Tyr Gln Leu Leu Pro Lys Asn
160 165 170

atc atg aag gcg gat ttt ttc cgg tat ttg gtg atc tac gcg cgc gga 579
Ile Met Lys Ala Asp Phe Phe Arg Tyr Leu Val Ile Tyr Ala Arg Gly
175 180 185 190

ggc acc tac tca gac atg gac acg gtg tgt tta aag ccg atc aag gac 627
Gly Thr Tyr Ser Asp Met Asp Thr Val Cys Leu Lys Pro Ile Lys Asp
195 200 205

tgg gcc acg ttt gat cgc gac ctg atc cac gct gcc gac aat aag gcc 675
Trp Ala Thr Phe Asp Arg Asp Leu Ile His Ala Ala Asp Asn Lys Ala

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210	215	220	
gat ctc tcc cag ata gat cca gaa gca aga acc acg cct gtg ggg ctg Asp Leu Ser Gln Ile Asp Pro Glu Ala Arg Thr Thr Pro Val Gly Leu 225 230 235			723
gtg att ggc att gag gcc gac ccg gac agg ccc gac tgg cac gag tgg Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp Trp His Glu Trp 240 245 250			771
ttc tgc cgc aga ctg cag ttc tgc cag tgg acg atc cag gcg aag ccg Phe Ser Arg Arg Leu Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro 255 260 265 270			819
gga cac ccg ctg ctg cgc gag ctg atc atc cgg atc gtg gag gag acg Gly His Pro Leu Leu Arg Glu Leu Ile Ile Arg Ile Val Glu Glu Thr 275 280 285			867
ttc cgc aaa cag cac atg ggc gtt ttg aaa aga gtg gaa ggc aag gac Phe Arg Lys Gln His Met Gly Val Leu Lys Arg Val Glu Gly Lys Asp 290 295 300			915
tgc ggc gca gat atc atg cag tgg aca gga ccg ggg ata ttt aca gac Ser Gly Ala Asp Ile Met Gln Trp Thr Gly Pro Gly Ile Phe Thr Asp 305 310 315			963
act ctg ttt gat tat ctg aac aat gtg gcg agc gac ggc aag ttg ggc Thr Leu Phe Asp Tyr Leu Asn Asn Val Ala Ser Asp Gly Lys Leu Gly 320 325 330			1011
gac ggg tac ggc gtg ggg tgc ttg tat tgg cgc aag cac ggc aaa tat Asp Gly Tyr Gly Val Gly Ser Leu Tyr Trp Arg Lys His Gly Lys Tyr 335 340 345 350			1059
aag ctg aaa aag aca gaa att aac aag aat aac gag cca ttg cat tct Lys Leu Lys Lys Thr Glu Ile Asn Lys Asn Asn Glu Pro Leu His Ser 355 360 365			1107
gag gac cag ctt atc aac tgg agg tgc ctg acc aac atg gac aag cca Glu Asp Gln Leu Ile Asn Trp Arg Ser Leu Thr Asn Met Asp Lys Pro 370 375 380			1155
aag atc atg ggc gac gta atg gtg tta cca atc acg agc ttt agt ccg Lys Ile Met Gly Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro 385 390 395			1203
aac gtg ggc cac atg ggc tca aag agc agc tca gat agg ctg gca ttt Asn Val Gly His Met Gly Ser Lys Ser Ser Ser Asp Arg Leu Ala Phe 400 405 410			1251
gtg gag cat tta ttt tct ggc agc tgg aag cca aaa aac aaa taggaaa Val Glu His Leu Phe Ser Gly Ser Trp Lys Pro Lys Asn Lys 415 420 425			1300
aataaataat tagctgcatt ttagataatt ctcatgagca ggcacagaac g			1351

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Lys Arg Ala Arg Leu Tyr Met Ala Thr Asn Arg Arg Leu Val Val Val
20 25 30
Leu Val Val Leu Leu Tyr Trp Val Val Gln Asn Val Trp Thr Trp Ser
35 40 45
Pro Gly Thr Arg Asp Leu Ala Gln Val Asp Ala Lys Ile Glu Ala Glu
50 55 60

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Leu Asn Ser Asn Leu His Thr Phe Gly Ala His Leu Arg His Leu Asn
 65 70 75 80
 Arg Leu Pro Ala Glu Ser Ala Thr Leu Arg Glu Lys Leu Thr Phe Tyr
 85 90 95
 Phe Pro Tyr Tyr Pro Glu Lys Pro Val Pro Asn Gln Ile Trp Gln Thr
 100 105 110
 Trp Lys Val Asp Leu Glu Asp Asp Asn Phe Pro Lys Gln Tyr Arg Arg
 115 120 125
 Phe Gln Lys Thr Trp Val Glu Lys Asn Pro Asp Tyr Val Tyr His Leu
 130 135 140
 Ile Pro Asp Ser Val Ile Glu Asp Phe Val Ala Ser Leu Tyr Ala Asn
 145 150 155 160
 Val Pro Glu Val Val Arg Ala Tyr Gln Leu Leu Pro Lys Asn Ile Met
 165 170 175
 Lys Ala Asp Phe Phe Arg Tyr Leu Val Ile Tyr Ala Arg Gly Gly Thr
 180 185 190
 Tyr Ser Asp Met Asp Thr Val Cys Leu Lys Pro Ile Lys Asp Trp Ala
 195 200 205
 Thr Phe Asp Arg Asp Leu Ile His Ala Ala Asp Asn Lys Ala Asp Leu
 210 215 220
 Ser Gln Ile Asp Pro Glu Ala Arg Thr Thr Pro Val Gly Leu Val Ile
 225 230 235 240
 Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp Trp His Glu Trp Phe Ser
 245 250 255
 Arg Arg Leu Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His
 260 265 270
 Pro Leu Leu Arg Glu Leu Ile Ile Arg Ile Val Glu Glu Thr Phe Arg
 275 280 285
 Lys Gln His Met Gly Val Leu Lys Arg Val Glu Gly Lys Asp Ser Gly
 290 295 300
 Ala Asp Ile Met Gln Trp Thr Gly Pro Gly Ile Phe Thr Asp Thr Leu
 305 310 315 320
 Phe Asp Tyr Leu Asn Asn Val Ala Ser Asp Gly Lys Leu Gly Asp Gly
 325 330 335
 Tyr Gly Val Gly Ser Leu Tyr Trp Arg Lys His Gly Lys Tyr Lys Leu
 340 345 350
 Lys Lys Thr Glu Ile Asn Lys Asn Asn Glu Pro Leu His Ser Glu Asp
 355 360 365
 Gln Leu Ile Asn Trp Arg Ser Leu Thr Asn Met Asp Lys Pro Lys Ile
 370 375 380
 Met Gly Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asn Val
 385 390 395 400
 Gly His Met Gly Ser Lys Ser Ser Ser Asp Arg Leu Ala Phe Val Glu
 405 410 415
 His Leu Phe Ser Gly Ser Trp Lys Pro Lys Asn Lys
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 <213> Artificial Sequence

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 <223> primer 168Not-N

<400> 3
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<210> 4
 <211> 39
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<220>
 <223> primer 168Not-C

<400> 4
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<210> 13
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<220>
 <223> primer for the amplification of alpha1,2-mannosidase in
 Aspergillus saitoi

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<210> 14
 <211> 69
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<400> 14

PCTA9407-2(한국생명공학연구원)

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cacccgcac 69